

free survival (DFS) and event-free survival (EFS). Eighty-two patients with stage II-IV *MYC*+ LBCL were treated with six cycles of R2CHOP. At end of treatment, 67% (95% Confidence interval [CI]: 58-75) of the patients reached CMR. With a median follow-up of 25.4 months, 2-year estimates for OS, DFS, EFS were 73% (95% CI: 62-82), 75% (95% CI: 63-84) and 63% change to: (95% CI: 52-73) respectively. In this prospective trial for newly diagnosed *MYC*+ LBCL patients, we found that administering R2CHOP was safe, and yields comparable CMR and survival rates as in studies applying more intensive chemotherapy regimens. Hence, these findings offer new prospects for *MYC*+ LBCL patients and warrant comparison in prospective randomized clinical trials. This trial was registered at [www.clinicaltrialsregister.eu](http://www.clinicaltrialsregister.eu) (#2014-002654-39).

## Introduction

Diffuse large B-cell lymphoma (DLBCL) comprises about 35% of all non-Hodgkin lymphomas (NHL) and is the most common lymphoma subtype.<sup>1</sup> The outcome of patients treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) is heterogeneous for which the IPI score and cell-of-origin (COO) are the most well-known denominators.<sup>2,3</sup> *MYC* rearrangement status is an independent prognostic factor, and is reported in 10-15% of DLBCL patients (hereafter *MYC*+ LBCL).<sup>4,7</sup> In about 30% of these patients, only a single *MYC* rearrangement is found (single hit [SH]), while in 70% *MYC* rearrangement is detected together with either a *BCL2* or *BCL6* rearrangement (double hit [DH]: *MYC*+/*BCL2*+ or *MYC*+/*BCL6*+ or with both (triple hit [TH]: *MYC*+/*BCL2*+/*BCL6*+).<sup>4</sup> It has been shown that in patients with *MYC*+ LBCL, standard first-line therapy with R-CHOP results in an inferior prognosis compared to those without *MYC* rearrangement (2-year OS 35% vs. 61%<sup>8</sup> and 5-year OS 31% vs. 66%<sup>6</sup>). Moreover, patients with *MYC*+ LBCL have an increased risk of central nervous system (CNS) relapse.<sup>5,6</sup> Recently, Rosenwald *et al.* demonstrated that the inferior prognosis of *MYC* rearranged patients is however largely observed in patients with DH/TH lymphoma.<sup>7</sup> In the revised World Health Organisation (WHO) 2017 classification, SH is not recognized as a separate entity in contrast to DH/TH lymphoma.<sup>1</sup>

In search for improvement, intensified chemotherapy regimens, such as hyper-CVAD and R-CODOX-M/R-IVAC, have been investigated. Data mainly come from sub-analyses of *MYC* rearrangement positive patients in trials designed for unselected DLBCL patients. These studies indicate that intensified treatment results in improvement of progression free survival (PFS), but not OS.<sup>9-11</sup> Only recently, a prospective, multicenter, single arm phase II study specifically designed for *MYC*+ LBCL patients showed that DA-EPOCH-R resulted in a promising CMR rate at end of treatment (EOT) of 74% and 4 year EFS and OS of 71% and 77%, respectively.<sup>12</sup>

Lenalidomide is an oral immunomodulatory drug with direct antitumor effects and indirect effects on the tumor microenvironment.<sup>13</sup> *In vitro* studies have demonstrated that lenalidomide exposure results in down-regulation of *MYC* and its target genes *via* cereblon and IRF4 in lymphoid cells, thereby providing the rationale for introducing lenalidomide as a therapeutic option in *MYC*+ LBCL.<sup>14</sup> Two phase II studies in ABC/non-GCB-subtype DLBCL have demonstrated that the addition of lenalidomide to R-CHOP (R2CHOP) is indeed feasible and may contribute to a favorable outcome by decreasing CNS relapse.<sup>15,16</sup> Against expectation, R2CHOP did not result in a survival advantage in ABC-subtype DLBCL, as has recently been shown in a phase III study (ROBUST).<sup>17</sup>

The present study reports the results of a prospective single-arm phase II trial for *MYC*+ LBCL patients treated with R2CHOP. Patients were identified through a nationwide molecular biomarker diagnostics program. We report outcome based on the primary endpoint, which was CMR by centrally reviewed a <sup>18</sup>F-fluorodeoxyglucose (<sup>18</sup>F-FDG) positron emission tomography (PET)-computer tomography (CT) scan at EOT, as well as 2-year OS, DFS and EFS rates.

## Methods

### Screening program and patient eligibility

To support timely diagnosis of *MYC*+ LBCL and optimal enrolment in the present clinical trial, a nationwide diagnostic support program for *MYC* rearrangement assessment by fluorescence *in situ* hybridization (FISH) was implemented.<sup>18</sup>

Patients  $\geq 18$  years with newly diagnosed DLBCL or with B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma (BCL-U) according to the WHO 2008 classification with a proven *MYC* rearrangement by FISH analysis including SH (not Burkitt lymphoma), DH or TH DLBCL were eligible.

During the screening period one cycle of R-CHOP, a short course of steroids, or irradiation to control local symptoms was allowed. Patients with Ann Arbor stage II-IV, a WHO performance status (PS) of 0-3,  $\geq$  one lesion of  $\geq 1.5$  cm on a contrast-enhanced CT scan and  $\geq$  one positive lesion on PET-CT scan were eligible.

Patients diagnosed with any other subtype of aggressive B-cell lymphoma, a history of follicular lymphoma, proven CNS localization or HIV positivity were excluded.

### Treatment

Treatment consisted of six cycles of standard R-CHOP every 3 weeks plus lenalidomide 15 mg orally on day 1-14 (R2CHOP; *Online Supplementary Table S1*), followed by two additional rituximab administrations.

Prophylactic intrathecal methotrexate or cytarabine ( $\geq 4$  administrations), pegfilgrastim, venous thromboembolism prophylaxis (with aspirin or low-molecular-weight-heparin), and *Pneumocystis* prophylaxis were mandatory.

### Safety assessments

Adverse events were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (AE), version 4.03. AE grade 1 were not reported.

### Study overview

This multicenter, phase II study was designed by investigators of HOVON and was approved by the medical ethics committee of the Amsterdam UMC. All patients provided written informed consent. The trial was conducted in accordance with the

Declaration of Helsinki and Good Clinical Practice guidelines. An independent data and safety monitoring board conducted a review during the planned interim analysis.

### Endpoints

The primary endpoint of the study was CMR on EOT PET-CT scan as determined by central review plus EOT bone marrow (BM) examination in case of BM localization at diagnosis. In case a BM examination was not repeated at EOT in patients with baseline BM localization and the EOT-PET scan showed no BM uptake localization, the response was classified as CMR (based on recent findings that CMR on PET-CT has a high negative predictive value for BM localization).<sup>23,24</sup>

Secondary endpoints were: OS defined as time from registration to death; DFS defined as time from achievement of first CMR on protocol until relapse or death whichever comes first; EFS defined as the time from registration to lack of CMR on EOT-PET-CT, relapse or death; and positive predictive value (PPV) and negative predictive value (NPV) of iPET-CT for EOT result.

### Statistical analyses

An optimal Simon two-stage design was used with a response rate of 45% as the null hypothesis, and 60% as the alternative hypothesis. With a statistical significance level of 5% and a power of 80%, the required number of patients was 77, with an interim analysis for futility involving the first 26 included patients. In order to overcome dropouts due to ineligibility, 85 patients were enrolled. All efficacy analyses were restricted to eligible patients, while safety analyses included all enrolled patients. Data cut-off was June 28, 2019.

For the clinical protocol, central pathology review, central PET-CT review and additional statistical information, see the *Online Supplementary Data*.

## Results

### Clinical characteristics

From April 2015 to February 2018, 85 patients were included from 20 hospitals in the Netherlands and Belgium. Three patients were declared ineligible (two because the MYC+ status was based on immunohistochemistry and not on FISH and one because of a transformed lymphoma), leaving 82 patients for efficacy and 85 patients for safety analyses.

Baseline patient and disease characteristics are shown in Table 1. The median age was 63 years (range: 28-82 years). 49 of 81 patients (60%) had a WHO performance status (PS) of 0; 58 of 71 patients (71%) had stage IV disease, and 42 of 82 patients (51%) had  $\geq 2$  extranodal localizations. The IPI score was high-intermediate and high in 65% of patients.

During treatment 12 of 82 patients went off protocol before completion (progressive disease [n=7], toxicity [n=2]; pulmonary embolism and diarrhea, other reasons [n=3]; new diagnosis of colon cancer, patient refusal, and vertebral fracture), see Figure 1.

### Pathology review

Diagnostic biopsy samples of all 85 patients were available for pathology review. Results of all 82 eligible patients are summarized in Table 1 and the *Online Supplementary Table S2*. A diagnosis of DLBCL according to the WHO 2008 classification was confirmed in 65 of 82 patients (79%) and BCL-U in 12 of 82 patients (15%) and

morphology was indecisive between DLBCL and BCL-U in 5 of 82 patients (6%). For classification according to the WHO classification 2017 see the *Online Supplementary Table S2*.

In 81 of 82 patients MYC rearrangement was confirmed at central review. Based on the intention to treat principle, the one patient in whom MYC rearrangement could not be confirmed was included in all analyses. In 9 of 82 cases, insufficient material was available to perform additional BCL2 and BCL6 rearrangement. In 73 of 82 cases, data on BCL2 and BCL6 rearrangement were available: 20 of 82 (26%) had a single MYC rearrangement (SH); 44 of 82 (54%) had DH lymphoma (31 patients had MYC/BCL2 rearrangements and 13 patients MYC/BCL6 rearrangements), and 9 of 82 (11%) had all three rearrangements (TH).

COO classification using a standard Hans algorithm showed GCB phenotype in 63 of 71 (89%) and non-GCB phenotype in 8 of 71 (11%). Lymph2Cx classification was performed in 38 cases showing GCB-subtype in 29 of 38 patients (76%), ABC-subtype in 7 of 38 patients (18%), and intermediate subtype in 2 of 38 patients (5%). Out of the 24 DH of TH patients, 21 showed GCB-subtype and 3 ABC-subtype. Out of the 12 SH patients, 8 showed GCB-subtype and 4 ABC-subtype.

### Treatment

Most patients (n=68) started with lenalidomide in the second cycle and continued lenalidomide for 14 days after the sixth cycle of R-CHOP. When MYC FISH results were available at diagnosis, R2CHOP was started in the first cycle (n=14) (Figure 1).

Patients received a median (interquartile range [IQR]) dose of the planned drugs in the R-CHOP regimen as follows: cyclophosphamide 99.9% (99.0-101); vincristine 100% (72.5-100); doxorubicin 99.5% (97.7-101); prednisone 100% (100-100); rituximab 98.1 (95.1-100); pegfilgrastim 100% (100-100). Lenalidomide was given at a median dose intensity of 100% (range: 85.7-100). 57 of 82 patients (70%) received the planned  $\geq 4$  intrathecal prophylactic administrations.

### Primary endpoint: CMR at EOT

At EOT PET-CT, 55 of 82 patients (67%) reached the primary endpoint of CMR (95% CI: 58-75,  $P < 0.001$ ), 5 of 82 patients (6%) reached a partial metabolic response (PMR), and 21 of 82 patients (26%) had progressive metabolic disease (PMD) (Table 2).

One patient went off protocol due to toxicity after cycle 5 without EOT PET-CT (response unknown).

Univariate logistic regression analysis of baseline characteristics (BM localization, WHO performance status, stage, B symptoms, IPI, number of extranodal sites and age) did not reveal any significant predictors for reaching CMR.

Exploratory descriptive subgroup analyses revealed no differences between SH and DH/TH patients regarding achievement of the primary endpoint: CMR rate in both groups was 70% and 66% respectively (nine patients with unknown BCL2 and BCL6 rearrangement not included).

### Secondary endpoints: survival analyses

With a median follow-up of 25.4 months (IQR 18.3-30.3), 1-year OS was 85% (95% CI: 76-91), DFS 77% (95% CI: 65-85) and EFS 66% (95% CI: 54-75). 2-year estimates for OS, DFS, EFS were and 73% (95% CI: 62-82),

75% (95% CI: 63-84) and 63% (95% CI: 52-73) respectively (Figure 2A-C).

Baseline patient characteristics (BM localization, WHO performance status, stage, B symptoms, IPI, number of extranodal sites and age) were not significantly predictive for prolonged OS in a univariate analysis at the 5% significance level.

Univariate regression analyses indicated that SH and DH/TH patients had comparable EFS and DFS, however DH/TH patients had a tendency for a higher risk of death compared to SH patients (Hazard ratio [HR] 4.18,  $P=0.055$ ; 95% CI: 0.97-18.02) (Online Supplementary Figure S1A-C). Separate analyses of DH *MYC/BCL2* and DH *MYC/BCL6* and TH in comparison to SH revealed no significant differences in OS (Online Supplementary Figure S2A-B).

In univariate analyses with response as time dependent covariate we found that patients who had achieved CMR at EOT PET experienced a reduced risk of death compared to patients who had not achieved CMR (HR 0.1, 95% CI: 0.03-0.33,  $P<0.001$ ), (Figure 3). EOT PET-CT predicted relapse within 12 months, with a positive predictive value (PPV) of 81% and a negative predictive value (NPV) of 93% (Online Supplementary Table S3A).

In total, 29 patients showed progressive disease (11 without achieving CMR, 18 after achieving CMR [(at interim or EOT PET-CT)] including one patient with a CNS relapse.

### Safety

Grade 2, 3 and 4 AE were seen in 27 (32%), 33 (39%) and 14 (16%) of all 85 registered patients respectively (Table 3). The most common grade 3-4 AE were neutropenia (18%), infections (14%) and gastrointestinal disorders (14%). Four patients experienced deep venous thrombosis (grade 2), and two patients pulmonary embolism (grade 3). Two of these patients (one with deep venous thrombosis and one with pulmonary embolism) had not received the mandatory thrombosis prophylaxis (protocol violation). One patient went off protocol due to grade 3 diarrhea.

71 serious AE were reported in 36 patients; 66 were due to hospitalization (42% infections, 26% gastrointestinal disorders), four to other conditions [two second primary malignancies, two recurrence of previously diagnosed (>5 year) malignancies]. One patient died during treatment due to progression. There were no treatment related deaths.

### Observational analysis: predictive value of iPET-CT

At iPET-CT after three cycles of R2CHOP, 57 of 82 patients (70%) were in CMR; of these 45 of 57 (79%) were still in CMR and 11 of 57 (19%) showed PMD at EOT PET-CT, and one missed EOT evaluation (Table 2). 23 of 82 patients (28%) were in PMR at iPET-CT; 10 of 23 (43%) of these converted to CMR, 4 of 23 (17%) remained in PMR, 9 of 23 (39%) showed PMD at EOT. The PPV of iPET-CT for predicting EOT PET-CT result was 60% (15 of 25), the NPV 79% (45 of 57) (Online Supplementary Table S3B).

### Discussion

From retrospective series it is clear that first-line R-CHOP therapy is not sufficiently effective for patients with *MYC*+ LBCL with CR rates of 40-50% and 3-year OS rates of 35% only.<sup>6,8</sup> Intensified chemotherapy regi-

mens such as R-CODOX-M/R-IVAC or autologous stem cell transplantation have not improved OS, and result in increased toxicity.<sup>5,9-11</sup>

We designed a prospective clinical trial for *MYC*+ LBCL patients, in which a time window of one cycle of R-CHOP was allowed to perform molecular diagnostics. This approach permitted high risk patients to start treatment immediately and overcame the bias of inclusion of mainly

**Table 1. Patient demographics and disease characteristics.**

	N	%
Patients completed treatment	82	100
Median age (range) in years	63 (28-82)	
Sex		
male	56	68
female	26	32
WHO performance status		
0	49	60
1	26	32
2	5	6
3	2	2
Prior treatment		
no	13	16
1 course of R-CHOP	68	83
only corticosteroids	1	1
Ann Arbor stage		
II	12	15
III	12	15
IV	58	71
Extranodal localisations		
0	31	38
1	9	11
≥2	42	51
LDH> ULN		
yes	57	70
no	20	24
unknown	5	6
Bone Marrow involvement		
yes	16	20
no	44	54
not done	22	27
IPI		
Low	12	15
Low-intermediate	17	21
High-intermediate	32	39
High	21	26
Morphology (WHO2008)		
DLBCL	65	79
BCL-U	12	15
indecisive between DLBCL or BCL-U	5	6
COO IHC (Hans classification)		
GCB subtype	63	77
Non-GCB subtype	8	10
Not evaluable	11	13
COO GEP (Nanostring) n=38		
GCB subtype	29	76
ABC subtype	7	18
Intermediate	2	5
FISH analysis		
single hit	20	24
double hit	44	54
<i>MYC</i> +/ <i>BCL2</i> +	31*	
<i>MYC</i> +/ <i>BCL6</i> +	13**	
triple hit	9	11
<i>MYC</i> + ( <i>BCL2</i> and <i>BCL6</i>	9	11
status unknown)		

Demographics and disease characteristics of 82 *MYC*+ LBCL patients treated with R2CHOP. LBCL: large B-cell lymphoma; DLBCL: diffuse large B-cell lymphoma; WHO: World Health Organisation; R-CHOP: rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone; FISH: fluorescence *in situ* hybridization, LDH: lactate dehydrogenase, ULN: upper limit of normal; GCB: germinal center B-cell subtype; COO: cell-of-origin; IHC: immune-histochemistry; GEP: gene expression profiling; BCL-U: B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma. \*from 4 of these patients *BCL6* status is unknown, \*\*from 1 of these patients *BCL2* status is unknown.